¹³C carbohydrate breath tests

R J Vonk, F Stellaard, H Hoekstra, H A Koetse

In paediatric practice proper digestion of carbohydrates is of major importance for adequate growth and development and for prevention of intestinal complaints. Several carbohydrates can be considered in this respect: (a) lactose: the main carbohydrate in the nutrition of early life and also in later life for many white people; (b) starch: the main energy supplier in our food; (c) fructose: a constituent of fruit juices and increasingly used as a sweetener; and (d) galactose: a component of lactose. The fate of this sugar will be considered in more detail in section five, dealing with the measurement of liver function.

Lactose maldigestion due to hypolactasia can be diagnosed in several ways. The non-invasive test most commonly used is the breath hydrogen test after lactose provocation. The reliability of this test, however, is disputed owing to non-H₂ producing flora,^{1,2} or extra intestinal influences that can disturb the test. Moreover, this test has to be done with unphysiological high doses of substrate to get a measurable hydrogen response in breath.^{3,4} This prompted investigators to develop diagnostic tools which permit the analysis of lactase enzyme activity non-invasively in a more direct way and therefore hopefully also in a more accurate and quantitative way. Hiele *et al* used naturally enriched ¹³C lactose and measured the ¹³CO₂ excretion in breath to diagnose adult hypolactasia patients.⁵

In the BIOMED programme SIGN this principle has also been used by Koetse *et al* (abstract 1) in paediatric patients. In 27 patients, lactase activity in jejunal biopsy specimens were compared with H₂ and ¹³CO₂ breath tests after administration of ¹³C lactose. Combination of the H₂/¹³CO₂ test results produced a higher sensitivity (85%) and a lower specificity (65%) than the outcome of both tests separately. The use of a combined H₂/¹³CO₂ breath test can reduce the number of jejunal biopsies needed for diagnosis of hypolactasia, without significant loss of discriminative power. The same group (Stellaard *et al*, abstract 2) further analysed the factors involved in the ¹³CO₂ excretion in breath after lactose administration. By comparing the ¹³CO₂ excretion rate after administration of ¹³C lactose, pre-digested ¹³C lactose, ¹³C glucose, and ¹³C galactose, the rate limiting step in ¹³C lactose use was investigated. Exercise increased four hours cumulative percentage dose recovered (cPDR) significantly. It was concluded that in healthy volunteers, glucose oxidation is the rate limiting step in the overall process of lactose use. In mild lactase deficient subjects, exercise shifts the rate limiting step to intestinal hydrolysis of lactose. In hypolactasic patients the rate limiting step is at the level of intestinal hydrolysis of lactose.

Fructose ingestion may lead to diarrhoea and abdominal pain in susceptible individuals. By means of a breath H₂ test, it has been shown that the absorption capacity of fructose is limited in adults⁶ as well as in children.^{7,8} Interestingly, both the percentage of malabsorbers and the peak breath H₂ increases were higher in toddlers compared with younger and older children. The relation of apple juice consumption to chronic non-specific diarrhoea (toddler's diarrhoea) has been well established and attributed to the high fructose content of apple juice. Fructose absorption is facilitated by equimolar doses of glucose and amino acids (especially L-alanine),⁹ the mechanism underlying this effect remains unclear. To study fructose absorption in a more direct way, Hoekstra combined breath H₂ studies with breath ¹³CO₂ studies (abstract 3).¹⁰ The results, however, did not clarify the mechanisms behind this stimulating effect of glucose and amino acids on intestinal fructose absorption. Variation in ¹³CO₂ output in breath can be caused by changes in oxidation rate, absorption rate or, in the case of colonic fermentation, colonic production rate. Without the knowledge of the rate limiting step in the overall utilisation process, the use of the ¹³CO₂ excretion rate in breath for mechanistic interpretation is difficult.

Starch digestion is affected by several factors. One of these is the physicochemical characteristics of the starch granules which can be influenced by pre-treatment such as cooking and heating and hydrolysis of the starch molecule by the enzyme "α-amylase". To monitor the digestion rate of corn starch, which is naturally enriched in ¹³C, the ¹³CO₂ breath test can be used, as first described by Hiele *et al*.¹¹ Especially in the study of the small intestinal digestion of resistant starch (Hylon VII and Novelose), this can be helpful to characterise the digestibility of the product as shown by Vonk *et al* (abstract 4). The six hour cPDR of raw Hylon VII and raw Novelose is 16.6 (SD 6.0) % and 15.5 (SD 6.0) % respectively. The six hour cPDR of glucose is 30.5 (SD 4.9) %. If we assume that 100% of the glucose is absorbed and the post-absorption metabolism of glucose from glucose itself or from starch is the same, then the six hour cPDR can be compared and used as an index of intestinal absorption. When these data for Hylon VII and Novelose are calculated in this way, both types of resistant starch are digested for 54% and 51% respectively in the small intestine. Colonic ¹³CO₂ excretion, which is defined as ¹³CO₂ output in breath which parallels H₂ excretion, starts after about eight hours, which implies that in the time period in which digestion is studied, colonic metabolism does not play a major part in ¹³CO₂ production.

Department of
Paediatrics, University
Hospital Groningen,
Groningen, the
Netherlands
R J Vonk
F Stellaard
H A Koetse

Department of
Paediatrics, Bosch
Medicentrum,
's-Hertogenbosch, the
Netherlands
H Hoekstra

Conclusion

^{13}C breath tests to measure carbohydrate assimilation are easy to use in clinical practice; a variety of them have been proposed and shown to work effectively. They are safe and non-invasive and thus can be used in any subjects. However, although the tests undoubtedly have potential, the present database with respect to breath test responses in health and disease is not large enough for specific "normal" or "abnormal" test outcomes to be described. Increasing the database must be a priority. A problem of special significance is that several factors other than nutrient digestion and absorption can influence the excretion of $^{13}\text{CO}_2$ in breath, and it is sometimes necessary to take care in the interpretation of the data. These problems will not be insurmountable especially if more research studies are done in which the overall metabolic pathways are studied simultaneously. For example, by combining the measurement of the appearance of ^{13}C glucose in plasma with breath tests will provide the information needed that should ultimately allow breath tests to stand by themselves as tools for routine diagnosis.

- 1 Gilat T, Ben Hur H, Gelman-Malachi E, *et al.* Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut* 1978;19:602–5.
- 2 Sategna-Guidetti C, Cruto E, Capobianco P. Breath hydrogen excretion after lactose and whole milk ingestion. *J Clin Gastroenterol* 1989;11:287–9.
- 3 Saavedra JM, Perman JA. Current concepts in lactose malabsorption and intolerance. *Annu Rev Nutr* 1989;9:457–502.
- 4 Bond JH, Levitt MD. Quantitative measurement of lactose absorption. *Gastroenterology* 1976;70:1058–62.
- 5 Hiele M, Ghos YF, Rutgeerts P, *et al.* $^{13}\text{CO}_2$ breath test using naturally enriched ^{13}C -lactose for detection of lactase deficiency in patients with gastrointestinal symptoms. *J Lab Clin Med* 1988;112:193–200.
- 6 Ravich WJ, Bayless TM, Thomas M. Fructose: incomplete intestinal absorption in humans. *Gastroenterology* 1983;84:26–9.
- 7 Kneepkens CMF, Vonk RJ, Fernandes J. Incomplete intestinal absorption of fructose. *Arch Dis Child* 1984;59:735–8.
- 8 Hoekstra JH, Kempen van AAWM, Bijl SB, *et al.* Fructose breath hydrogen tests. *Arch Dis Child* 1993;68:136–8.
- 9 Hoekstra JH, Aker van den JHL. Facilitating effect of amino acids on fructose and sorbitol absorption in children. *J Pediatr Gastroenterol Nutr* 1996;23:118–24.
- 10 Hoekstra JH, Aker van den JHL, Kneepkens CMF, *et al.* Evaluation of $^{13}\text{CO}_2$ breath tests for the detection of fructose malabsorption. *J Lab Clin Med* 1996;127:303–9.
- 11 Hiele M, Ghos YF, Rutgeerts P, *et al.* Measurement of the rate of assimilation of oligo and polysaccharides by $^{13}\text{CO}_2$ breath test and isotope ratio mass spectrometry. *Biomed Mass Spectrom* 1988;16:133–5.

(1) Detection of low intestinal lactase activity in children by use of a combined $^{13}\text{CO}_2/\text{H}_2$ breath test

H A KOETSE, F STELLAARD, C M A BIJLEVELD, H ELZINGA, R BOVERHOF, ¹R VAN DER MEER, R J VONK
Department of Paediatrics, Laboratory of Nutrition and Metabolism, University Hospital Groningen, the Netherlands, and ¹Netherlands Institute for Dairy Research (NIZO), Ede, the Netherlands

To reduce the number of small bowel biopsies needed for diagnosis of hypolactasia in paediatric patients with specific gastrointestinal complaints, a combined $\text{H}_2/^{13}\text{CO}_2$ breath test was performed after administration of ^{13}C lactose. The aim of the study was to diagnose hypolactasia non-invasively with a higher accuracy than with the H_2 breath test, which is known to have a high proportion of false negative results. To validate this new test it was used on 27 patients who had to undergo small bowel biopsy. In

this patient group lactase activity was reduced (<10 U/g protein) in 13 cases, the sensitivity and specificity of the H_2 test were 54% and 90%, of the $^{13}\text{CO}_2$ test 69% and 70%. False negative results did not always occur in the same patients. In five out of six patients with both breath tests positive, lactase activity was low. In 13 out of 15 occasions with both tests negative, lactase activity was normal. In six out of 12 cases with only one of both tests positive, lactase activity was low.

The following clinical regimen is proposed: if the results of both breath tests point in the same direction (that is, $+/+$ or $-/-$) no biopsy specimen is taken. In case of conflicting results ($-/+$ or $+/-$) a biopsy specimen is taken. In the population studied this leads to a reduction of the number of biopsies to 64% with confirmation of diagnosis in 91% (30/33) by measured lactase activity.

Conclusion: The use of a combined $\text{H}_2/^{13}\text{CO}_2$ breath test can reduce the number of jejunal biopsies needed for diagnosis of hypolactasia, without significant loss of discriminative power. This test is therefore well suited for diagnosis in patients with aspecific gastrointestinal complaints and for population prevalence studies of hypolactasia.

(2) Rate limiting step in lactose use in humans. Consequences for the diagnosis of mild lactase deficiency

F STELLAARD, H A KOETSE, H ELZINGA, R BOVERHOF, A KLIMP, D VEGTER, J LIESKER, R J VONK
Department of Paediatrics, Laboratory of Nutrition and Metabolism, University Hospital Groningen, the Netherlands

In the present study, the steps involved in the use of lactose—that is, digestion, monosaccharide absorption, galactose metabolism, and glucose oxidation—were studied in healthy adults by analysing breath $^{13}\text{CO}_2$ excretion after consumption of 40 g ^{13}C lactose, predigested ^{13}C lactose, ^{13}C glucose, or ^{13}C galactose. The effect of increased glucose oxidation was measured under exercise conditions (bicycling, 50 Watt) applying 80 g ^{13}C lactose. No difference was observed in the $^{13}\text{CO}_2$ excretion curves after administration of glucose, predigested lactose, and lactose. The four hour cumulative percentage doses recovered in breath (four hours cPDR) were 20.3 (SD 4.5) %, 19.2 (SD 5.5) %, and 19.9 (SD 4.9) % for glucose, predigested lactose, and lactose respectively. Exercise increased four hours cPDR significantly: 66.1 (SD 6.2) % v 19.6 (SD 3.9) %. One subject (A) had a mild non-symptomatic lactose malabsorption indicated by a positive H_2 breath test at rest and during exercise. The $^{13}\text{CO}_2$ breath test of subject A at rest was indistinguishable from that of the others (four hours cPDR 16.6 v 19.6 (SD 3.9) %), whereas the test was clearly positive during exercise (four hours cPDR 20.5 v 66.1 (SD 6.2) %).

Conclusion: In healthy volunteers, glucose oxidation is the rate limiting step in the overall process of lactose use. In mild lactase deficient subjects, exercise shifts the rate limiting step to intestinal hydrolysis of lactose.

(3) Use of ^{13}C breath test in fructose malabsorption

H HOEKSTRA
Department of Paediatrics, Bosch Medicentrum, 's-Hertogenbosch, the Netherlands

Fructose ingestion may lead to diarrhoea and abdominal pain in susceptible individuals. Fructose absorption is facilitated by equimolar doses of glucose or amino acids (L-alanine): the mechanism underlying this effect remains unclear. To study intestinal fructose absorption in a more direct way we combined breath H_2 studies with $^{13}\text{CO}_2$ studies.

Gastric emptying was studied using L-glycine-1-¹³C in children, aged 12.1–16.0 years. After 25 g of fructose and 27.5 g of glucose, when given together, gastric emptying was significantly ($p < 0.05$) slower than with either sugar alone. In a second series of experiments five children, aged 12.0–15.9 years, were tested with 25 g of fructose, alone and with equimolar doses of glucose and L-alanine. Four younger children, aged 3.1–6.1 years, were tested with 2 g/kg (maximum 37.5) of fructose, alone or with an equimolar dose of L-alanine. All fructose solutions were enriched with 15 mg of D-fructose-¹³C-6. In all nine children, fructose was malabsorbed as judged by breath H_2 increases 20 ppm, and the addition of glucose or L-alanine resulted in significantly lower breath H_2 increases ($p < 0.005$ for glucose, $p < 0.001$ for alanine). In contrast, the addition of alanine or glucose did not change the pattern of breath ¹³CO₂ excretion in the five older children, whereas in the four younger children (with relatively higher doses) L-alanine addition resulted in significantly lower increases in breath ¹³CO₂. In the group with four younger children, for each time point breath H_2 and ¹³CO₂ concentrations after fructose were compared with those after fructose plus L-alanine; in 20 out of 24 points both H_2 and ¹³CO₂ were higher after fructose. These results suggest that ¹³CO₂ not only originated from the oxidation of absorbed substrate but also, at least in part, from colonic bacterial metabolism.

(4) Digestion of resistant starch

R J VONK, R DE GRAAFF, S TABAK, ¹YUE-XIN YANG, F STELLAARD

Department of Paediatrics, Laboratory of Nutrition and Metabolism, University Hospital Groningen, the Netherlands, and ¹Institution of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing, China

To increase the colonic availability of short chain fatty acids, resistant starch can be used. Part of this resistant starch is digested in the small intestine, the residue spills over into the colon. To develop a monitoring system of starch digestion, we compared the metabolic fate of three types of starch: custard (25% amylose, 75% amylopectin), Hylon VII (62% amylose, 38% amylopectin) and Novelose (62% amylose, 38% amylopectin).

The three types of starch are of corn origin and therefore naturally enriched in ¹³C. After administration of 40 g of starch to seven healthy volunteers, CO₂ and ¹³CO₂ excretion rate in breath were analysed each 30 minutes during six hours. Administration of 40 g of ¹³C glucose and analysing ¹³CO₂ response was done as a control and reference experiment. In three healthy volunteers ¹³CO₂ and H_2 response were measured during 26 hours after Hylon VII consumption. ¹³CO₂ was measured by isotope ratio mass spectrometry (IRMS). After administration of 40 g of the (raw) substrate the six hours cPDR was 30.5 (SD 4.9) % (glucose), 32.8 (SD 2.7) % (custard), 16.6 (SD 6.0) % (Hylon VII) and 15.5 (SD 5.9) % (Novelose). In the ¹³CO₂ response roughly two phases can be distinguished: <six hours, which is due to intestinal absorption, and >eight hours, which parallels H_2 production and is presumably of colonic origin.

Assuming that glucose is totally absorbed by the small intestine, the ¹³CO₂ response in breath after glucose consumption can be used as a reference for starch digestion. Comparing these data it can be calculated that starch is digested in the small intestine completely (custard), or partly: 54% (Hylon VII) and 51% (Novelose). These data show that the ¹³CO₂ response in breath can be used as a monitoring system for starch digestion of ¹³C enriched starch.